Proceeding booklet summarizing the main conference of WGs

(*by María Ortiz-Bueno, Javier Molina Estévez and Karim Benabdellah*)

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INTRODUCTION

This document presents a comprehensive overview of meetings held by the working groups within the COST Action CA21113. Therefore, its aim is to provide a detailed account of the collaborative efforts and progress made by each working group, highlighting the key discussions, decisions, and outcomes from their respective sessions. These meetings serve as a platform for fostering collaboration, exchanging insights, and ensuring the alignment of efforts towards the overall goals of the Action. Therefore, in this document it will be summarized how COST Action CA21113 brings together a diverse network of experts, researchers, and stakeholders with the shared objective of advancing research and innovation in the field of genome editing to treat human diseases (**GenE-HumDi**).

Figure 1 below illustrates a timeline with the sequence of meetings conducted by the different working groups over the past year (since October 2023). It provides a chronological overview of key sessions, highlighting the frequency and duration of these collaborative efforts. This visual representation aims to offer a clear and comprehensive understanding of the progress and milestones achieved by each working group, demonstrating the coordinated efforts and ongoing commitment to advancing the objectives of the Action throughout the year.

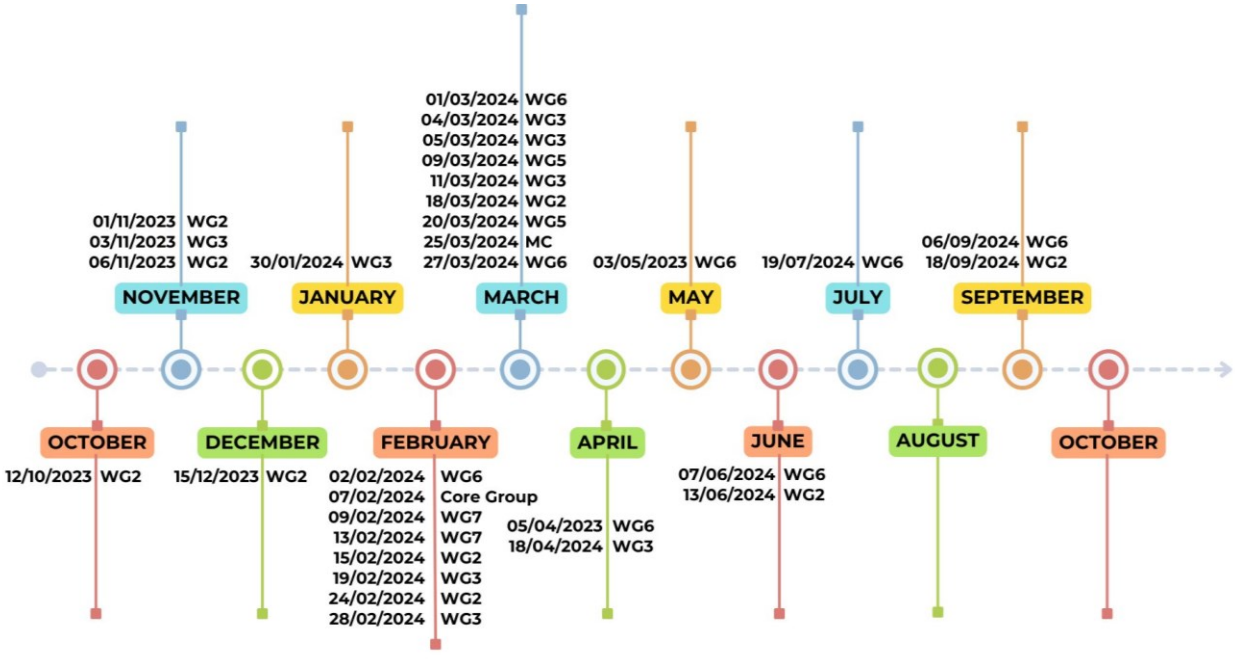

Figure 1. Meetings of the different WGs since October 2023.

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WG2. IMPROVEMENT OF GE TECHNOLOGIES.

This Working Group, headed by Dr Rasmus O. Bak from the university Aarhus focused on consolidating all information related to efficacy and specificity of various genome editing (GE) tools, with the aim of establishing most suitable tools and research priorities in the field.

MAIN TASKS AND ACTIVITIES

1. To map the existing endonuclease-Independ platforms (EIP) and their potential output in different applications. This specific task will be opened to include researchers from third countries (especially the USA) to ensure more up-to-date information on the state of the art of this technology.
2. To map the existing endonuclease-depend on platforms and their potential output in different applications.
3. Create multidisciplinary networks to work on specific objectives identified in objective 1.
4. Consolidate research platforms through grant applications to national, European and international financial bodies/funders.

DESCRIPTION OF DELIVERABLES AND TIMEFRAME

- Reference documents (including efficacy, specificity and catalogue of producers) of the available endonucleases independent GE tools.
- Reference documents (including efficacy, specificity and catalogue of producers) of the available endonucleases and new developments.
- To establish research priorities required to advance GE into clinic.
- Reviews and Co-authored papers on at least 2 model gene editing approaches.

The timeline illustrated by figure 2 outlines the meetings organized within Working Group 2 (WG2) since October 2023. It provides a chronological overview of the collaborative efforts between the different members of this WG.

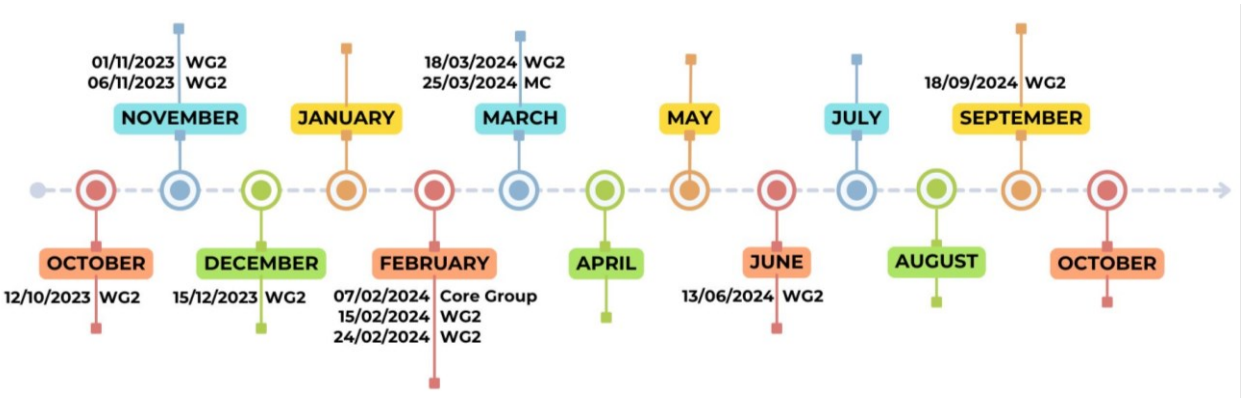

Figure 2. Meetings of WG2 since October 2023.

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WG2 MEETING MINUTES - 12/10/2023

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín
MEETING TITLE: WG2 General Meeting
PARTICIPANTS ATTENDING: 22 people
DURATION OF THE MEETING: 39 minutes 15 seconds
PARTICIPANTS LIST:

1. Rasmus O. Bak
2. Francisco Martín
3. Davide Seruggia
4. Alessandro Michienzi
5. Claudio Mussolino
6. Veronika Viktorija Borutinskaitė
7. Laura Torella
8. Michael Schmück-Henneresse
9. Urszula Oko
10. Peter Nielsen
11. Ayla Wyninckx
12. Serif Senturk
13. Omer Aydin
14. Pavlovic Guillaume
15. Ciaran Lee
16. Manuel F. V. Goncalves
17. Eivind Dale Valen
18. Julian Ceron
19. Alfredo Silva
20. Stefan Seemann
21. Julian Grünewald
22. Carles J. Ciudad

AGENDA (A) (Objectives and meeting points):

- Welcome to new members and short introduction of GenE-HumDi
- GenE-HumDi activities and general announcements
- WG2 deliverables
- WG2 activities and coordination of these
- Q&A and any other business

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AGREMENTS ADOPTED

<table>
<tr><th>Agenda Point</th><th>Decision made</th></tr>
<tr><td>Welcome to new members and short introduction of GenE-HumDi</td><td>➢ WG2 co-leader, Rasmus Bak, presented himself and new members were welcomed.
➢ The concept of COST actions was presented along the specific GenE-HumDi purpose.</td></tr>
<tr><td>GenE-HumDi activities and general announcements</td><td>➢ An overview of GenE-HumDi recent activities was presented and members were encouraged to apply for upcoming STSM and ITC grants.
➢ New call for virtual mobility grants was mentioned and members encouraged to apply.
➢ Annual conference was announced to take place in Cyprus in the week beginning April 8th. Members were encouraged to participate and mark their calendars.</td></tr>
<tr><td>WG2 deliverables</td><td>➢ General WG2 mission and deliverables were presented.</td></tr>
<tr><td>WG2 activities and coordination of these</td><td>Specific suggestions for activities in WG2 were presented.

A call for papers was made. There is co-financing available (up to €3,000) for an open access paper from a group of WG2 members (see requirements in box below). If interested, WG2 members should send an email to WG2 leaders specifying which action members will contribute to suggested article, the target journal, and a short description of the scope and aim of the article which must fall within the aim of WG2. Deadline for this suggestion is December 1st, 2023. Note that the article must be peer-reviewed and the journal should belong to the first three quartiles Q1-Q3 of articles ranked by the SCImago Journal Rank (SJR).

<table>
<tr><th>Dissemination or Communication Product</th><th>List of eligible expenses</th><th>Specific Conditions</th></tr>
<tr><td>Scientific publication in Open Access
Proof of delivery:
If Accepted (or in Press), but not published yet: accepted final manuscript in pdf format + proof of acceptance by the publisher.

If Published: final publication in pdf format</td><td>• Open Access fee for scientific publications
• Proof reading, editing, translation and layout expenses.</td><td>• Be the result of the work of the Action.
• And
• Be authored by
○ Action participants from at least 3 different COST Member.
Or
○ Action participants from at least 2 different COST Member and 1 NNC.
Or
○ Action participants from at least 2 different COST Member if resulting from an STSM funded by the Action.</td></tr>
</table>

A database will be made that list WG2 members, areas of expertise, resources that can be shared, interest in STSM hosting, etc. This task is ongoing an more info will follow when this has been set up and members will be invited to input their information in the database.</td></tr>
</table>

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	As a general activity for the action, a webinar series is planned. There will be 1-2 webinars per year where presenters will be from WG2. Laura Torella presented suggested activities for young researcher members of WG2. She will organize online meetings for them once per semester. The first two meeting will be on the following dates: YRM1 29th November 2024, 15:00 CET YRM2 14th March 2024, 15:00 CET YRM3 October '24, TBT YRM4 March '25, TBT Invitations will follow shortly. All WG2 members should encourage young researchers in the labs and network to participate. In addition, two more activities for young researchers were proposed: (1) Focused mentoring sessions with more senior scientists, and (2) educational online meetings/webinar with training sessions on topics like 'designing a good experiment', 'the most up-to-date advances in GE technologies' or 'bioinformatic and statistics'. Such activities would require one or two more people in the work coordinating this. The format for WG2 general meetings was presented. The general agenda will be: 1. Announcements (news, meetings, activities, COST funding opportunities, etc). 2. WG2 activities and coordination of these. 3. Agenda items from WG2 members. 4. Select WG2 members present themselves. Short format, max. 6 min total, 3-4 speakers. 5. Any other business. Reg. point 4, a sign-up link will be set up and sent to all WG2 members that can express their interest to present themselves and their research. There were no objections to the proposed activities for WG2.
Q&A and any other business	For the funds covering open access fee, how are these paid out to the institution responsible for the article submission? Response: This cost is reimbursed following a claim with proof of delivery (publication PDF or letter with proof of acceptance) and an invoice. Regarding STSMs, is there any possibility that these can be longer than the current 4 weeks. This would be beneficial to the possible scope of visitors' project as well as the hosting lab/institution, and longer STSMs might encourage more applications. Response: STSMs are in fact not limited in the possible duration, but the maximum grant size is €4,000, which sets a natural limitation to the duration of a STSM. It was suggested to have WG2 activities in conjunction with other conferences. Others mentioned that some of the WG2 members in the meeting were attending the upcoming ESGCT conference in Brussels (October) and the EMBO workshop in Seville (November). Response:

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	We will take this into consideration and encourage action members to connect at these two upcoming meetings. For the next WG2 general meeting in December, it was suggested to avoid Dec. 6., 7., and 8. due to holidays in Spain. Response: Next general meetings will be on following dates (calendar invitations will follow shortly): GM3 Dec. 15th 2023 14:00 CET GM4 Feb. 15th 2024 14:00 CET GM5 COST conference, Cyprus, April 2024 GM6 June 13th 2024 14:00 CET GM7 September '24, TBT GM8 November '24, TBT

WG2 MEETING MINUTES - 15/12/2023

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: WG2 General Meeting

PARTICIPANTS ATTENDING: 20 people

DURATION OF THE MEETING: 48 minutes

PARTICIPANTS LIST:

1. Rasmus Bak
2. Francisco Martín
3. Ciaran Lee
4. Miguel A. Moreno-Mateos
5. Karolina Skvarova Kramarzova
6. Lucie Peterkova
7. Lluis Montoliu
8. Laura Torella
9. Alba Olaso Llorca
10. Michael Schmück-Henneresse
11. Giedrius Gasiunas
12. M.F.V Goncalves
13. Asma Ressaissi
14. Claudio Mussolino
15. Guillaume Pavlovic
16. Julian Ceron
17. Alexander Brennan
18. Stefan
19. Veronika Borutinskaite
20. Lydia Teboul

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AGENDA (A) (Objectives and meeting points):

- Welcome
- Presentation from three WG2 members:
  - Miguel A. Moreno-Mateos
  - Claudio Mussolino
  - Lluis Montoliu
- GenE-HumDi activities and general announcements
- WG2 deliverables & activities
- Q&A and any other business

AGREMENTS ADOPTED

Agenda Point	Decision Made
Welcome	WG2 co-leader, Rasmus Bak, welcomed both old and new members of WG2.
Presentation of three members	Three WG2 members gave a presentation on themselves, their research focus, and potential contributions, collaboration, ideas/needs within the COST network. These presenters were: ➢ Miguel A. Moreno-Mateos ➢ Claudio Mussolino ➢ Lluis Montoliu Following presentations, there were questions from the audience. Rasmus informed that there are still open time slots for upcoming WG2 general online meetings in February and June 2024.
GenE- HumDi activities and general announcements	The concept of COST actions and general funding opportunities were briefly presented to new WG2 members along the specific GenE-HumDi purpose. An overview of GenE-HumDi recent activities was presented and members were encouraged to participate inupcoming virtual conference concerning the short-term scientific missions from funding Year 1 on Dec. 19th. Everyone was reminded and encouraged to participate inthe annual conference to take place in Cyprus in the weekbeginning April 8th.
WG2 deliverables &activities	The general WG2 mission and deliverables were presented. A call for articles was repeated. There is co-financing available (up to €3,000) for open access papers from a group of WG2 members (see requirements in box below).If interested, WG2 members can send an email to WG2 co-leaders specifying which action members are suggested to contribute to the article, the target journal, and a short description of the scope and aim of the article which must fall within the aim of WG2. Note that the article must be peer-reviewed,andthe journal should belong to the first three quartilesQ1-Q3 of articles ranked by the SCImago Journal Rank (SJR).

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	<table><tr><th>Dissemination or Communication Product</th><th>List of eligible expenses</th><th>Specific Conditions</th></tr><tr><td>Scientific publication in Open Access Proof of delivery: If Accepted (or in Press), but not published yet: accepted final manuscript in pdf format + proof of acceptance by the publisher. If Published: final publication in pdf format</td><td>• Open Access fee for scientific publications • Proof reading, editing, translation and layout expenses.</td><td>• Be the result of the work of the Action. • And • Be authored by ○ Action participants from at least 3 different COST Member. Or ○ Action participants from at least 2 different COST Member and 1 NNC. Or ○ Action participants from at least 2 different COST Member if resulting from an STSM funded by the Action.</td></tr></table> To meet the two deliverables on two reference documents of available endonuclease-independent and -dependent genome editing tools, a Mentimeter questionnaire will bemade. The leadership is currently working on this and hopes to send it out to WG2 members before Christmas. The results from this questionnaire will be the basis of a report that will constitute the reference documents. It was reiterated that a webinar series will be establishedfor the entire COST action, which will likely include the presentation from 1-2 WG2 members per year. The outcome of the young researcher meeting held on Nov. 28th was presented by leader of the WG2 young researcher (YR) network Laura Torella. She presented an overview of the general agenda and main outcomes. Moreover, she emphasized that among the activities discussed and identified by YRs, there was a collective expression of interest in participating in educational webinars focusing on the key expertise areas of WG2. Notably, the YRs alsoexpressed a desire to attend webinars hosted by expertsfrom other working groups. This approach aims to broaden their expertise, facilitate collaborations, encourage internships, and advance their research careers. Following discussions with the Action Chair, Vice Chair, andthe Scientific Communication Coordinator, the initiative was positively valued and considered interesting for the entire YR GenE-HumDi community. Consequently, YRs from all working groups have been contacted to announce a series of educational webinars accessible to young researchers across all groups. A list of proposed webinars covering the key expertise areas of each working group has been presented. We highly value the opinions of our YRs, and the most selected topics will be featured in our webinar sessions, led by experts from our COST action. General agenda of WG2 online meetings was presented and a reminder that the next meeting will be onFeb. 15th at 14:00 CET. Invitations will be sent out before Christmas.
Q&A and any other business	No questions were raised.

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WG2 MEETING MINUTES - 15/02/2024

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: WG2 general meeting
PARTICIPANTS ATTENDING: 29 people
DURATION OF THE MEETING: 50 minutes
PARTICIPANTS LIST

1. Rasmus O. Bak
2. Francisco Martín
3. Veronika Viktorija Borutinskaitė
4. Ayla Wyninckx
5. Alba Olaso Llorca
6. Alessandro Michienzi
7. Gonzalo Martínez Navajas
8. Karolina Skvarova Kramarzova
9. Lucie Peterkova
10. Seruggia Davide
11. Ilknur Yılmaz
12. Ciaran Lee
13. Carla Fuster
14. Bernhard Schmierer
15. Tao Wang
16. Fisnik
17. Karim Benabdellah
18. Pedro J. Real
19. Joan Pera García
20. Asma Ressaissi
21. Ceron Madrigal, Julian
22. Michael Schmück-Henneresse
23. Dimitra Micha
24. Peter Nielsen
25. Alberto Malerba
26. Claudio Mussolino
27. Asma Ressaissi
28. Peter Nielsen
29. Lorea Blázquez

AGENDA (A) (Objectives and meeting points):

- Welcome
- GenE-HumDi activities and general announcements
- WG2 deliverables
  - Update on Mentimeter questionnaire
- GenE-HumDi general meeting in Cyprus (Francisco Martín)
- Short introduction of WG2 members (6 min each)
  - Francisco Martín Molina
  - Gonzalo Martínez Navajas
  - Lorea Blázquez
- Q&A and any other business

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AGREEMENTS ADOPTED

Agenda Point	Decision made
Welcome	WG2 co-leader, Rasmus Bak, welcomed both old and new members of WG2 and shortly introduced the action and WG2 mission. There have been a few structural changes in the action leadership. The new structure is depicted in the slide deck.
GenE-HumDi activities and general announcements and the GenE-HumDi general meeting in Cyprus	A call for short-term scientific missions (STSMs) will be announced shortly (within weeks). Please consider if these could be an opportunity for international networking. A Mentimeter questionnaire was run in Dec. '23 / Jan '24. This has now been finished and the WG2 leadership is drafting a reference document that provides an overview of WG2 members and research interests. This document will be distributed when finalized and covers the first two deliverables of WG2. The 3rd deliverable of WG2 is "To establish research priorities required to advance GE into clinic.". This will be addressed at the second annual meeting in Cyprus in April. A previous call for papers for co-funding by the action for open access has been finalized and one proposal was submitted. This is pending final approval from the action leadership. Webinar series of the action has been kicked off and has confirmed speakers. Karim Benabdellah gave a brief overview of the second annual action meeting in Cyprus in April. WG2 co-leader Paco Martin gave an overview of activities within WG2 where specifically four different topics will be discussed at a meeting on March 4th. An overview of these discussions will be presented at the meeting in Cyprus. The young researcher network has outlined research topics of interest and will be looking into organizing educational webinars on select topics. The next meeting in the young researcher network is scheduled for March 14th.
Presentation from three WG2 members	Three WG2 members gave a presentation about themselves, their research focus, and potential contributions, collaboration, ideas/needs within the COST network. The three presenters were: ➢ Francisco Martín Molina ➢ Gonzalo Martinez Navajas ➢ Lorea Blázquez After presentations, there were some questions from audience. There are still open time slots for the upcoming WG2 general online meeting on June 13th, 2024.
Q&A and any other business	No questions were raised.

WG2 MEETING MINUTES - 24/02/2024

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: WG2 Kick-off Meeting

PARTICIPANTS ATTENDING: 16 people

DURATION OF THE MEETING: 59 minutes 23 seconds

PARTICIPANTS LIST:

1. Nuri Öztürk
2. Micha, D. (Dimitra)
3. Claudio Mussolino
4. Miguel Moreno-Mateos
5. Schmück-Henneresse, Michael
6. Rasmus O. Bak
7. Francisco Martín Molina
8. Goncalves, M.F.V. (CCB)
9. Giedrius Gasiunas
10. Julian Ceron
11. Guillaume Pavlovic
12. Gal Cafri
13. Peter E. Nielsen
14. Lydia Teboul
15. Cristina Salado Manzano
16. Pedro J. Real

AGENDA (A) (Objectives and meeting points):

- Presentation of the COST action and MoU.
- Presentation of WG2 with emphasis on deliverables.
- Presentation of WG2 members.
- Meeting in Granada and planning future meetings.
- Suggestions on activities in WG2 and meeting deliverables.
- Q&A and any other business.

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AGREMENTS ADOPTED

Agenda Point	Decision made
Suggestions on activities in WG2	➢ Create a list of participants of WG2. Potentially using Google Doc? Possible on Gene-Humdi website? ➢ This list could be part of a list of other WGs. The list should contain: ➢ Main expertise areas ➢ List of techniques, reagents and protocols thatcan be shared ➢ Link to the research group's AddGene site(plasmid sharing) ➢ Others
Suggestions on activities in WG2	Contribute to a special issue in a journal. Based on the list above, groups could be formed from same areas of expertise, and they could come together and write for example a review article or protocol to be published.
Suggestions on activities in WG2	Physical meetings can be held in conjunction with international conferences, e.g. ESGCT potentially as hybrid meeting that allows non-conference participantsto participate online. This will reduce number of travels,travel costs, CO2 emission, etc.
Suggestions on activities in WG2	Establish a communication channel, e.g. Slack or Teams.We should see what possibilities are there and vote. This channel could be used to: ➢ Share information on meeting/conference participation where WG2 members could meet upand discuss collaborations/network. ➢ Ask general questions on genome editing, protocol requests, availability of reagents, cell lines, etc. Identify knowledge/expertise gaps in WG2 and reach out to other WGs to get an overview of expertise in these.
Q&A and any other business	➢ Still time to apply for the training course in Granada? ➢ Request: please increase notification time for scheduling meetings and courses.
Next steps	1. Send out PDF of the meeting's presentation including minutes. (Rasmus) 2. Identify time for next meeting, possibly physical meeting in conjunctionwith ESGCT '23 (Rasmus & Paco). 3. Execute the decisions made above (Rasmus & Paco). 4. To provide signed commitment letters.

WG2 MEETING MINUTES - 18/03/2024

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: Kick-off Meeting by Google Meet

DURATION OF THE MEETING: 56 minutes

4 challenges IN WP2 TO BE DISCUSSED IN FEBRUARY:

1. **Challenge 1: Improving efficacy, specificity, and safety (ESS) of new GE tools.**

Discussion Points. Comparison of the ESS of different platforms to achieve similar goals:

- **Gene disruption (Base editors, PRIME, CRISPR (NHEJ)**
- **Gene correction (Base editors, PRIME)**
- **Gene insertion (PRIME, Cas9 Transposases)**
- Dimitri: Cargo limit is higher up to 10kb (Cas9)

Outcomes:

- Consensus on best versions for each new GE tools for **gene disruption:**
- Hig fidelity Cas9: eSpCas9 (addgene #79145) SpCas9-HF1 (#72247), SpCas9- HF4 (#72249).
- Base editors (RB). Highly efficient and quite safe (very low off target). ABEmax-NRTH (Addgene #136922).
- Advice on the best platforms for **Gene correction:**
- Base editors (RB). Highly efficient and quite safe (very low off target). ABEmax-NRTH (Addgene #136922).
- DSB-based HDR. *See challenge 2*.
- Poor expertise on PRIME edition of participating groups. Action: search for other groups in the consortium that may have use PRIME editors.
- Advice on the best platforms for **Gene insertion**.
- Poor expertise on PRIME edition of participating groups. Action: search for other groups in the consortium that may have use PRIME editors. More efficient for small insertions and corrections.
- DSB-based HDR. *See challenge 2*.
- New technologies: FiCAT gene writing platform. Based on Cas9 and PiggtBac transposase could be an alternative for large DNA insertions.
- **Dr Dimitri Ivancic** will lead the discussion in Cyprus.

2. **<u>Challenge 2: Improving efficacy of HDR/Repair.</u>**

Discussion Points. Strategies to improve:

- **Gene insertion/gene correction -** HDR based.
- **Gene insertion/gene correction -** PRIME based.

Poor expertise on PRIME edition of participating groups. Action: search for other groups in the consortium that may have use PRIME editors.

Outcomes:

- Consensus on the best strategies to improve HDR in each cell type (T cells, HSCs, MSCs, liver, etc).
- **Influence of delivery tools** on the HDR efficacy: coordinate with WP3
- Use of **small molecules** to enhance HDR versus NHEJ. Pros and cons.
- Advice for the best strategy to achieve gene correction: compare PRIME versus HDR for different applications.
- Poor expertise on PRIME edition. Absence of data comparing different tools for specific applications. Action: search for other participants in the cost action that have experience of PRIME edition and partners to do a side-by-side comparison.
- **Dr Claudio Mussolino** will lead the discussion.

3. **<u>Challenge 3: To avoid immune responses to GE tools (Julien).</u>**

Discussion Points.

- ***In vivo* approaches: Immune responses to delivery vehicles (viral versus non-viral)**
  - Modifications of the vector.
  - Modifications of the route of delivery.
  - Modification of the host.
- ***Ex vivo* approaches**: Cell responses to delivery vehicles and/or GE tools. To be discussed in WP3. Action: talk to Yonglun Luo and Karim Benabdellah to include this point in his session.

Outcomes:

- Consensus on best strategies to reduce immune responses to viral vectors.

- Modifications on methodology to produce retroviral vectors (absence of FBS) can reduce immune responses.
- Engineering of the capsids to reduce immunogenicity of viral particles.
- Consensus on immunogenicity of viral versus non-viral vectors.
- Non-viral vectors could be a better approach to avoid immune responses for in vivo strategies. Not clear that this applies to **all** non-viral vectors. Cannot compare at least they reach equivalent efficacies.
- Consensus on cell responses to the different vectors and advice of the method of choice for immune cells versus non-immune cells.
- Important to consider the innate response of immune cells to select the delivery method and the use of RNA, DNA or RNPs.
- Selection of the person in charge of presenting these results at Cyprus meeting (10min). Dr Julien Ceron will lead the discussion in Cyprus.

Challenge Description: The first GE therapy has already been approved for beta-thalassemia and sickle cell disease. However, there are still several limitations to translate into clinical applications other GE strategies. The aim of this discussion will be to find the main limitations that GE face to reach approval of new ATMPs for different indications.

Discussion Points:

- Indications close to market authorization or to Phase II/III: which GE strategies apply?
- Cas9 (NHEJ).
- Base Editing.
- CAR-T, Inherited disorders.
- Efficacy versus safety and price: when is a good balance to get approval by FDA or EMA.
- Is scale up a main limitation for translation?

Outcomes:

- List of GE strategies in advanced stages of clinical applications.
- List of main limitations of actual GE strategies to reach market authorization.
- Propose candidates to write a review/report on the limitations of GE strategies to advance into clinic.
- Selection of the person in charge of presenting these results at Cyprus meeting (10 min).

WG2 MEETING MINUTES - 13/06/2024

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: WG2 General Meeting

PARTICIPANTS ATTENDING: 21 people

DURATION OF THE MEETING: 60 minutes

PARTICIPANTS LIST:

1. Rasmus O. Bak
2. Francisco Martín
3. Carles J. Ciudad
4. Ángeles
5. Enola Missonnier
6. Ceren Sahin
7. Laura Torella
8. Isabel Ortuño Lizaran
9. Claudio Mussolino
10. Natalia Martínez Gil
11. Karolina Skvarova Kramarzova
12. Ciaran Lee
13. Julian Ceron Madrigal
14. Nicolás Cuenca
15. Julien Valton
16. Alessandro Michienzi
17. Carla and Xavier
18. JC Segovia
19. Pedro Lax
20. Joan Pera Garcia
21. Lucie Peterkova

AGENDA (A) (Objectives and meeting points):

- Welcome
- GenE-HumDi activities and general announcements WG2 deliverables
- Young Researcher Network (Laura)
- Short introduction of WG2 members (6 min each): Carlos J. Ciudad, Karolina S. Kramarzova and Isabel O. Lizaran
- Q&A and any other business

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AGREEMENTS ADOPTED

Agenda Point	Decision made
Welcome	The WG2 leader, Rasmus Bak, welcomed both old and new members of WG2 and shortly introduced the action and WG2 mission. Please see attached slide deck.
GenE-HumDi activities and general announcements and the GenE-HumDi general meeting in Cyprus	A training school at the National Institute of Chemistry, Slovenia is scheduled for June 26-28. This is organized by Dusko Lainscek and Roman Jerala with this title: 'Comprehensive understanding of the latest advancements in gene editing delivery methods'. Another training school organized by Yonglun Luo at Aarhus University is scheduled for Aug. 26-28. The title is 'Methods for *in vivo* CRISPR delivery covering LNP and viral methods'. The action expects to fund 10 travel scholarships. The cost action was presented by Karim Benabdellah at the CRISPR Medicine Conference in Copenhagen, Denmark in April and by Lluis Montoliu at the annual congress of the European Forum of Farm and Animal Breeders in Bologna, Italy in June.
WG2 deliverables	Third deliverable of WG2 is "To establish research priorities required to advance GE into clinic.". This was addressed at the second annual meeting in Cyprus in April. Two review articles will be written to cover this. Interested potential authors should get in touch.
Young Researcher Network	Laura Torella informed about the recent initiative on a webinar series for the Young Researcher Network, in collaboration with CRISPR Medicine News. Everyone is welcome to attend, and the first two webinars have been a great success: ➢ May 27th, 'Strategies and Tools for *Ex Vivo* and *In Vivo* Genome Editing', invited speaker: Marc Guell WG2-3 ➢ June 11, 'CRISPR Clinical Trials: Current Progress and Future Perspectives in *Ex Vivo* Approaches', invited speaker Annarita Miccio WG3-5). Next webinar is by Paula Río (WG3-5) on June 25th, 3pm CEST 'CRISPR Clinical Trials: Current Progress and Future Perspectives in *In Vivo* Approaches'. There is a call for speakers for the next three seminars with the topics: ➢ 'Viral and Non-Viral Delivery Vectors in Gene Editing' ➢ 'Beyond DNA Modification: RNA Editing' ➢ 'Immunological Responses to Cas' Please consider if you can be a speaker or if you have any suggestions for speakers and contact Laura. The webinar lasts 1

	hour, featuring a 45–50-minute presentation followed by a 10-minute Q&A session. Young Researchers can also enjoy a one-on-one chat opportunity at the end of the webinar, limited spots are available.
Presentation from three WG2 members	Three WG2 members gave a presentation about themselves, their research focus, and potential contributions, collaboration, ideas/needs within the COST network. These presenters were: ➢ Carlos J. Ciudad ➢ Karolina S. Kramarzova ➢ Isabel O. Lizarán (Nicolas Cuenca lab) Following the presentations there were questions from the audience. There are open time slots for the upcoming WG2 general online meeting on September 18th and November 20th 2024.
Q&A and any other business	No questions were raised. Next WG2 meeting will be on September 18th and November 20th, 2024 at 14:00 CEST.

WG2 MEETING MINUTES - 18/09/2024

ORGANISING ENTITY: Working Group 2, Rasmus Bak & Francisco Martín

MEETING TITLE: WG2 General Meeting

PARTICIPANTS ATTENDING: 34 people

DURATION OF THE MEETING: 56 minutes

PARTICIPANTS LIST:

1. Rasmus O. Bak
2. Ester López Aguilar
3. Jose Carlos Segovia Sanz
4. Mostafa Hossam Mahmoud M Khairy
5. Basma Naiisseh
6. Joan Pera García
7. Francisco Martín
8. Cris Eguizabal
9. Melissa Whitehead
10. Claudio Mussolino
11. Ciaran Lee

12. Julian Ceron Madrigal
13. Alessandro Michienzi
14. Karolina Škvárová
15. Giedrius Gasiūnas
16. Serif Senturk
17. Alba Olaso Llorca
18. Laura Torella
19. Dimitrie Ivancic Djermanovic
20. Pilar Puig
21. Veronika Viktorija Borutinskaitė
22. Lorea Blázquez
23. Petros Patsali
24. Gal Cafri
25. Álvaro Plaza Reyes
26. Irene S.
27. Sibtain Haider
28. Ozgue Dogus Erol
29. Ivan Hernandez - Countagen
30. Álvaro Plaza Reyes
31. Alfredo Silva
32. Marija Tomovic
33. Roman Jerala
34. Kulbhushan Sharma

AGENDA (A) (Objectives and meeting points):

- Welcome
- GenE-HumDi activities and genera announcements
- Young Researcher Network (Laura)
- WG2 deliverables
- Short introduction of WG2 members (6 min each):
    - Dimitrije Ivancic Djermanovic
    - Sunil Martín
- Q&A and any other business

AGREEMENTS ADOPTED

Agenda Point	Decision Made
Welcome	WG2 co-leader, Rasmus Bak, welcomed both old and new members of WG2 and shortly introduced the action and WG2 mission.
GenE-HumDi activities and general announcements	4 STSM grants are expected to be announced soon (expected November 2024). The annual action meeting for the 3rd grant period will be held in parallel with the CRISPR Medicine Conference in April in Copenhagen, Denmark. One training school is ongoing at Aarhus University with the topic: Clinical Applications of In Vivo Gene Editing: Methods, Challenges, and Solutions. Another workshop is being held in Barcelona from Oct. 3-4th: Coupling Genome Editing Tools with Delivery Systems. Four planned training schools for the next grant period: ➢ Omics Approaches in Gene Editing (Granada, Spain) ➢ Gene Editing: From Tools to Therapeutic Applications (Modena, Italy) ➢ Advanced Delivery Systems in Gene Editing: Exploring Lipid Nanoparticles and Extracellular Vesicles (Freiburg, Germany) ➢ Off-target Analysis (proposed by WG4, TBD) Members were encouraged to stay updated on activities on the GenE-HumDi website: https://www.genehumdi.eu/
Young Researcher Network	Laura Torella informed the audience of the Young Researcher (YR) activities currently taking place. She highlighted the GENOME EDITING CHAT Webinars, organizedby and for YRs in collaboration with CRISPR Medicine News. She reminded the expert audience about the opportunity to present at these educational webinars on topics such as genome editing delivery, Cas9 immunity, and RNA editing, suggested by our YRs, but other topics are welcomed. GenE-HumDi is funding up to 8 active YRs to attend the ESGCT conference and present abstracts, offering them a chance to have *one-on-one* meetings with genome editingexperts at the conference to discuss their research and future career perspectives. She reported suggestions from the YR community requesting an easier way to connect with other YRs from their Action. Rasmus suggested creating a LinkedIn or similar profile. This

	topic will be discussed further withthe dissemination group. Laura concluded with a final *call-to-action* reminder for YRs from Inclusiveness Target Countries (ITC) to apply for ITC supporting grants to attend conferences. Finally, she reminded us that everyone, including YRs, could present our COST action at international conferences and have the chance to be financially supported.
WG2 deliverables	Final formal delivery of WG2 are two papers on model gene editing approaches. One paper is being prepared on genetic engineering of CD34+ HSCs. Another isin the works, initiated by Alfredo Silva. WG2 members were encouraged to get in contact with Rasmus by email before September 27th for possible contribution to this if the expertise aligns.
Presentation fromthree WG2 members	Dimitrije Ivancic gave a presentation about himself, his research activities, and the research focus of Dr. Marc Guell´s group. Sunil Martín was also scheduled to present but was not present. After the presentation, there were questions from audience. There is still one open time slot for the upcoming WG2 general online meeting on November 20th, 2024 at 14:00 CEST. Invitations have been sent out. Please get in touch with Rasmus if you have not received the invitation.
Q&A and any other business	No questions were raised.

WG3. DELIVERY STRATEGIES.

This Working Group is focused on evaluating *ex vivo* delivery systems and identifying the optimal delivery method for each cell type and genome editing (GE) technology. Moreover, this WG assesses *in vivo* delivery systems to determine the most effective method for each animal model and route of administration.

MAIN TASKS AND ACTIVITIES

1. Generating a map of the current delivery methods used for GE of the different cell types that are used for clinical applications. To date non-viral systems, based on ribonucleoparticles (RNP) or plasmids, are the *ex vivo* systems of choice to deliver the GE complex into the target cells. The RNP or plasmids could be transfected or electroporated into cells according to the cell type. Therefore, efficacy and specificity of GE of the different GE tools delivered by different methods will be compared and best systems for each cell type identified.
2. Generating a map of the current delivery methods used for in vivo GE in different animal models and in clinical settings. Including comparison of efficacy and specificity data for different GE tools delivered by different methods in each tissue/organ of interest.

DESCRIPTION OF DELIVERABLES AND TIMEFRAME

- Reference document with a consensus on the best GE tool/delivery method combination for each cells type.
- The development of a consensual handbook describing those cell types where further improvements are required to achieve efficient and specific GE.
- Reference document with a consensus on the best GE tool/delivery method combination for each targeted tissue of the different animal models and patients.
- State-of-the-art manuscript describing current delivery technology that appears to have promise in the field.

WG3 has been divided into several specific subgroups, each focusing on distinct areas of the group's objectives. Regular meetings have been held for each of these subgroups to ensure progress, collaboration, and alignment with the overall goals of the working group.

The timeline depicted in figure 3 presents the different meetings held by Working Group 3 (WG3) over the past year. This chronological summary seeks to enhance comprehension of the collaborative endeavors and notable accomplishments attained by WG3 during this timeframe.

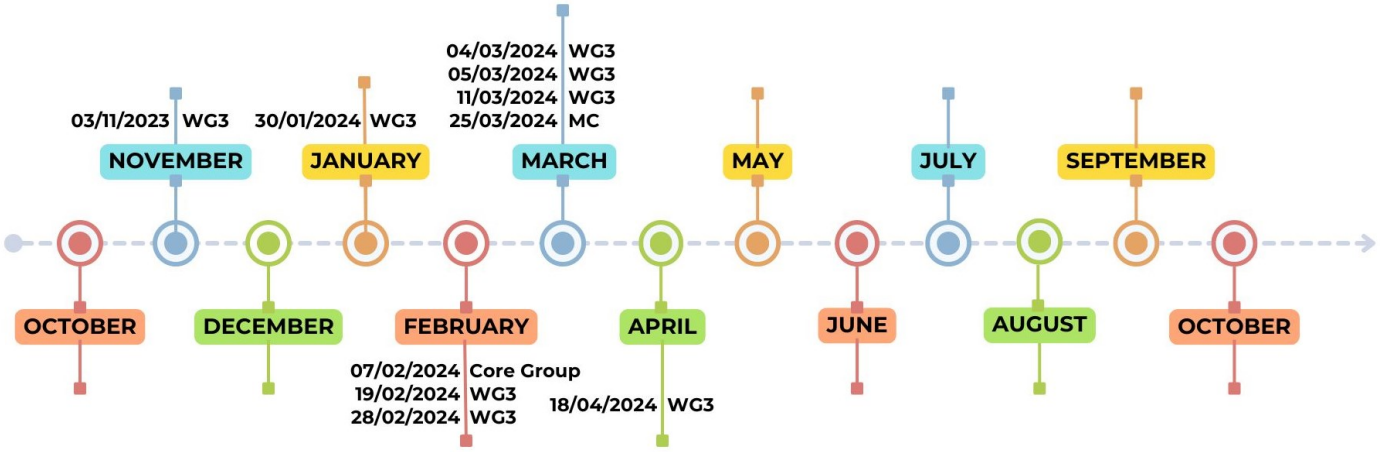

Figure 3. Meetings of WG3 since October 2023.

WG3 MEETING MINUTES - 03/11/2023

Unfortunately, the minutes from these meetings are not available.

WG3 (*HSCs* Subgroup) MEETING MINUTES - 30/01/2024

MODALITY: Kick-off Meeting by Zoom

AGENDA

1. Monthly/bimonthly meetings to facilitate discussions on the respective cell types, sharing insights and planning collaborative efforts.

2. Suggest/Discuss topics with a particular focus on the challenges associated with working with HSC and GE, following the guidelines outlined in our MOU. These discussions may lead to:

   Identifying of potential speakers for our event in Cyprus within the WG3/HSC.

   - Handbook of protocols for gene editing in different cell types including HSCs.

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- Publication of a special issue (specific to each cell type?)

PARTICIPANTS LIST (10 attendees)

1. Annarita Miccio
2. Carsten Lederer
3. Francisco Martín Molina
4. Karim Benabdellah
5. Alessia Cavazza
6. Rajeevkumar R. Nair
7. Ayal Hendel
8. María Ortiz Bueno
9. Manuel Sánchez Martín
10. Loubna Mazini

DISCUSSION TOPICS

1. Optimization of CRISPR-Cas9, Talen, ZFN Delivery: Explore and compare various methods for efficient delivery into HSCs, including viral vectors, electroporation and nanoparticles.

2. Enhancement of Editing Precision: investigate strategies to improve the specificity of gene editing in HSCs, minimizing off-target effects and enhancing precision.

3. Long-Term Effects of Editing: investigate the long-term effects of gene editing on the self-renewal capacity and differentiation potential of HSCs (which animal models?)

4. Combination Therapies: explore the potential for combining gene editing techniques with other therapies to enhance engraftment and efficacy in clinical applications.

ACTIONS

- **Goals of WG3** discussion and agreement about sharing minutes, slides and list of attendees of the first meeting.
- **Potential speakers of Cyprus meeting**. It was highlighted that novel delivery methods should be discussed. Jose Segovia will discuss the preclinical studies to move to the clinics a CRISPR/Cas9 strategy based on the use of HSCs. He is also exploring novel delivery methods for in vivo gene editing, but he will already give one talk, so this option was excluded. Alessia Cavazza was also invited to give a talk on in vivo foetal gene therapy. Furthermore, novel delivery methods could also be discussed by Rosario Sanchez Martin that is working with Francisco Martin Molina on nanoparticle delivery of gene editing tools. Carsten Lederer and Loubna Mazini are also working on VLP (e.g., nanoblades) or NP delivery with so far limited or inconsistent results. However, Alessia Cavazza said that there will not be a specific talk from WG3 but on day 2 there will be a 1-hour session to discuss 4 challenges. WG3 leaders will probably clarify this point.
- **Sharing protocols** could be envisioned. Agreement about the creation of a common folder on the website.
- **Potential review to be proposed on HSCs** (but the invoice should be paid by OCT 2024). In addition, potential contributors should be identified (e.g. Ayal Hendel, Alessia Cavazza, Annarita Miccio, Carsten Lederer)
- **Delivery methods in HSCs** used by the WG3 members (excel file could be shared on the website).
- **Publication of special issue.** € 3,000 allocated to WG3 for Open Access publication. Short proposals should be sent to karim.benabdel@genyo.es describing:
    - COST action members that will contribute
    - Summarize the content/scope of the article (must fall within WG3 theme)
    - Previous proposal deadline 15 dec 2023

The members collectively agreed to take the following actions:

- Next topics will be NP delivery in HSCs- Rosario Sánchez Martín could be invited to briefly introduce the different NP types that could be used for this purpose. Other potential contributors? Sharing data also negative?

WG3 (*T-cells Delivery* Subgroup) MEETING MINUTES - 19/02/2024

Unfortunately, the minutes from this meeting are not available.

WG3 (*HSCs* Subgroup) MEETING MINUTES- 28/02/2024

MODALITY: Kick-off Meeting by Zoom

AGENDA

- Updates on WG3-HSC group and actions (Annarita Miccio)
  - Next meetings
  - Sharing protocols
  - Review
- Discussion topics: NP delivery in HSCs (Rosario Sánchez Martín)
- Open discussion

PARTICIPANTS LIST (20 attendees)

1. Alessia Cavazza
2. Annarita Miccio
3. Ayal Hendel
4. Carsten W Lederer
5. Francisco Martín Molina
6. Ikram Salih
7. Giorgia Santilli
8. Javier Molina
9. José-Carlos Segovia
10. Karim Benabdellah
11. Ling Peng

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12. Loubna Cell
13. María Ortiz Bueno
14. Mario Amendola
15. Mégane Brusson
16. Michaela Semeraro
17. Paula Río
18. Rajeevkumar Nari Rave
19. Rosario Fernández
20. Rosario Mª Sánchez-Martín

DISCUSSION TOPICS

- Updates on WG3-HSC group and actions (Annarita Miccio) Next meetings
- Sharing protocols
- Review
- Discussion topics: NP delivery in HSCs (Rosario Sánchez Martín)
- Open discussion

ACTIONS

The members collectively agreed to take the following actions:

- Organize bimonthly meetings that will be registered and shared with WG3-HSC participants. We could have Invited speakers and volunteers discussing positive /negative data.
- Handbook of protocols for gene editing in different cell types including HSCs. We will coordinate with WG3 leaders and other subgroups in Cyprus.
- Publication of a special issue (specific to each cell type?). We will discuss in Cyprus with WG3 leaders.
- Discussion Topics: we will share the slides of Rosario Sánchez Martín and propose next topics based on today's discussion.

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Open discussion

Christina Eich could give other updates on PLGA.

Major problem to solve de-targeting the liver.

- Main organs targeted: liver and spleen, S. Rivella recently showed liver toxicity.
- How to de-target the liver
    - Maybe changing the lipid composition of the LNP to prevent liver targeting.
    - Use biodegradable NP that are not toxic for the liver.
    - Intra-bone injection could be feasible even if not ideal (e.g., used in a clinical trial in Milan for beta-thal patients) and could prevent liver toxicity.
        ▪ The NP could still go somewhere even if injected in the BM (but probably less than with iv delivery). Moreover, intra-bone vs intravenous cell injection in mice => not really very clear/specific improvement (NGS mice with human cells-Jose Carlos Segovia).
    - Fetal gene therapy (abstract Naldini's group using LV in fetuses) could be a solution, but at this stage there are hepatocytes so the risk is that the NP will also target liver cells.
    - Using a microRNA enabling liver de-targeting (Rivella's paper).
    - Conclusions: try to identify technology/tools/solutions that enable liver de-targeting.

Paper with Cre, did they discuss the CNS? Risk of CNS targeting?

We are using LNPs to edit HSPCs, but which assays should we run to evaluate toxicity?

- CFC assay and transplantation to verify toxicity are common for HSCs but are there other assays?
- Immune response (genes activated by RNA stimuli) as done for electroporation?
- Rosario will share protocols from the National Institute on Cancer, such as cytokine/interleukin quantifications and pre-clinical characterization.
- There are protocols to evaluate the general toxicity of NP, but we should perhaps select specific tools/protocols for HSCs.

Other concern: the expression (and the MFI) of the modified/edited genes could differ from the antibody clones used and could maybe impact the results/conclusions or the follow-up of patients.

- We should maybe select a specific clone to have comparable results.

Conclusions:

- Comparing NPs and other tools to target HSCs and de-target the liver.
- Immune responses and toxicity should be evaluated using common protocols.

WG3 (*Non-Viral Delivery Methods* Subgroup) MEETING MINUTES- 04/03/2024

TIME: 1:00 pm (Spain)

MODALITY: Virtual meeting by Google Meet

PARTICIPANTS LIST (15 attendees)

1. Dusko Lainscek (*co-leader*)
2. Loubna Mazini (*co-leader*)
3. Karim Benabdellah (*WG3 co-leader*)
4. Carsten Lederer
5. Paula Río
6. Peng Ling
7. Virginia Arechavala-Gomeza
8. Carla Giacomelli
9. Arístides López-Márquez
10. Lydia Álvarez-Erviti
11. Nina Kostevšek
12. Diego Balboa
13. Inês Pinto
14. Diana Campillo
15. María Ortiz

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AGENDA

- Welcome and introduction of participants
- Brief recap of the Action and purpose of the WG3
- Aims of the working subgroup: Non-Viral Delivery Methods
- Discussion about new future meeting topics
- Meeting conclusions

DISCUSSION

The first meeting of the Non-Viral Delivery Methods working subgroup began with the individual presentation of each of the 18 participants. The co-leaders of the subgroup began, followed by each of the participants. From this interaction it was concluded that, in summary, the members of this subgroup are working on gene editing strategies to correct rare diseases, such as Fanconi anemia and collagen 6 muscular dystrophies, using a variety of methods such as CRISPR-Cas9, base editing and lipid nanoparticles, both *invitro* and *in vivo*. There is a common interest in finding the best way and strategy to deliver gene correction tools effectively to meet the needs of the diseases being treated.

During the second part of the meeting, Dusko focused on commenting on the objective of this subgroup within WG3, taking WG3 as a reference. Therefore, he commented that the main aim is to basically develop guidelines or to basically drive our vast knowledge. It's basically to establish very efficient delivery systems that could work *ex vivo* for different cell types, for different gene editing tools, and that would work also *in vivo*. Then, the aim would be identifying basically the best method for each cell type and animal model. In other words, to generate a map of the current delivery methods used for gene editing tools for different cell types that are used for clinical applications that can be translated from cell types to different animal models in order to deliver different tools by different methods that can specifically target different tissue or organ of interest.

On the other hand, they highlighted the importance of standardized methods of delivering non-viral genomic editing tools through collaboration and sharing of positive and negative results to improve efficacy in different cell types and animal models. They seek to establish specific protocols, foster collaboration, and joint proposal writing, as well as identify constraints and potential speakers for future meetings and events.

Furthermore, Dusko commented that a CRISPR delivery workshop will be organized at the National Institute of Chemistry Ljubljana, Slovenia from June 28thto 30th, focusing on LNP preparation, characterization, and methods for targeted delivery.

For Loubna, it's a good way to send the global survey to large people from the WG3and try to collect the maximum information about how they are managing their research. She highlighted that the goal is to understand the type of particle used for delivery and the methodology employed in research, with the aim of translating research findings to clinical applications. Furthermore, she also emphasizes the importance of considering criteria for disease correction and sharing limitations within the research group to avoid wasting time, resources, and money. The survey mentioned will provide valuable information for analysis and decision-making regarding the most efficient delivery methods. Additionally, it acknowledges that not everyone has the capability to synthesize certain particles, such as LNP.

At the end and as co-leader of Working Group 3, Karim expressed gratitude to Dusko and Loubna for their involvement in a survey within the Working Group. They also discuss the importance of collaboration and coordination among subgroups to draft potential papers or reviews.

CONCLUSIONS

- Suggestion for active participation of all members of this working subgroup.
- Agreement on the creation of a survey.
- Agreement on the creation of the survey commented.
- Suggestion about discussing the potential benefits and expertise of working on biological non-viral vectors (such as nanoparticles and exosomes) as most people in the subgroup are working on this.
- Suggestion of using the COST website to connect people interested in testing different models or showcasing their skills.

WG3 (*T-cells Delivery* Subgroup) MEETING MINUTES- 05/03/2024

MODALITY: Kick-off Meeting by Google Meet

AGENDA

- Welcome to this new working subgroup within WG3.
- Results of the mentimeter.
  - Participants
  - Tools
  - Applications
- Thoughts/input on Challenges for T cell delivery to discuss in Cyprus.
- Confirmation of the next meeting slot.
- Questions.

PARTICIPANTS LIST (8 attendees)

1. Francisco Martín Molina
2. Karim Benabdellah
3. Begoña Diez Cabeza
4. Diana Campillo
5. Alessandra Recchia
6. María Ortiz Bueno
7. Tommaso Ferrari
8. Loubna Mazini

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DISCUSSION TOPICS

- Agreement on ¾ brief talks each month/two months.
- Date the first three talks (after Cyprus meeting): Karim, Paco and Tommaso Ferrari.
- Agreement of the meeting in Cyprus.
- Reference document to be delivered to WP3 leaders.
- Review article: New tools for the delivery of Genome editing tools into T cells.

WG3 (*Liver & Hepatocytes Delivery* Subgroup) MEETING MINUTES- 11/03/2024

MODALITY: Kick-off Meeting by Google Meet

PARTICIPANTS LIST (5 attendees):

1. Lourdes Ruiz Desviat (working subgroup leader)
2. Mario Amendola
3. Alex Garanto
4. María Ortiz Bueno
5. Álvaro Somoza

Nerea Zabaleta wrote later to explain she could not join due to the different time zone as she is in the US.

AGENDA AND DISCUSSION

Brief round of introductions, in which each participant explained their interest and their expertise in liver/hepatocyte delivery, some groups are experts in gold or albumin-based nanoparticles (A. Somoza), others in AAV (A. Amendola, N. Zabaleta) and others work in metabolic diseases with liver as target organ (A. Garanto & LR Desviat).

Exposition of the objectives: exchange ideas/protocols and tools for liver/hepatocites delivery and to get to know each other for potential STSM to learn specific methods (e.g. AAV production). There is limited expertise in *in vivo* liver delivery among participants.

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Need to coordinate with other subgroups/WG regarding writing a review or organizing webinars. As for a potential joint review with other subgroups/working groups, this is programmed to be discussed during the next general meeting in Cyprus.

Ideas for webinar series: this could be open to everyone, not only for COST Action members, depending on the Zoom/teams license), and thus form part of the dissemination objectives. In this sense, registration with a Google formular or similar is needed, as COST will ask for the number of participants, from which country, etc. The idea is to schedule seminars by members of the subgroup, e.g. Álvaro Somoza to give a talk about their nanoparticles or to invite expert researchers working on gene editing:

- Sabine Fuchs (prime editing in vivo, has conducted clinical trials, to be contacted by A. Garanto).
- Lombardo (epigenetic editing, see Nature 2024 PMID: 38418872, to be contacted by M. Amendola).
- G. Schwank (AAV and intern-split base editors for liver, to be contacted by LR Desviat).

These webinar series can be monthly, with each month to be organized by a different subgroup, to make it more general and interesting to everyone. This is to be discussed and organized with Action coordinators.

WG3 (*CNS & Muscle Delivery* Subgroup) MEETING MINUTES- 18/04/2024

TIME: 11:00 AM (Spain)

MODALITY: Kick-off Meeting by Google Meet

PARTICIPANTS LIST (11 attendees)

1. Lorea Blázquez (working subgroup co-leader)
2. Cecilia Jiménez (working subgroup co-leader)
3. Rajeevkumar Nair (working subgroup co-leader)
4. Karim Benabdellah (WG3 co-leader)
5. María Ortiz Bueno
6. Lydia Álvarez-Herviti
7. Alicia Rodríguez

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8. Ana del Pozo
9. Mª Ángeles Solinís
10. Peng Ling
11. Dusko Lainscek

AGENDA

- Introduction to this working subgroup and its objectives.
- Individual presentation of each member.
- Organization of the working subgroup, next meetings and suggestions.
- Creation of a template.

DISCUSSION

First meeting of the muscle and brain subgroup for COST CA21113 Action where Cecilia as co-leader started the meeting with a brief introduction of the objectives of the meeting.

And then we went for a round of introductions for each of the 11 attendees. So Lydia Álvarez-Herviti from Rioja spoke about her work on extracellular vesicles for delivering to the brain, in particular for Parkinson's disease. Karim said he was interested in nanotechnology, mainly for hematological disorders and he had worked on shRNA. Peng from the Institute of Neuroscience in France in CERN, she said they have a nanoformulation. Therefore, they have nanopectors, which they have already used successfully to deliver siRNA, mRNA.

Lorea Blázquez as co-leader of this working subgroup, she said that her group was interested in RNA misprocessing, and that she had been working for several years on the Cas13 systems. She asked Karim how restrictive this subgroup was to gene editing, and whether it could be also included to the types of nucleic acids. Karim said that, of course, provided we accomplish the deliverables most people have only started working on gene editing. And we can incorporate our expertise working with other nucleic acids. Rajeev from Norway said that they are interested in how to deliver transgenes and gene editing components, as well as hRNA calcium sensors in different cell types.

Later, Dusko talked about his work on cartesans.
And he also said that they are developing a therapeutic strategy for a neurological disease caused by beta-catenin deficiency. He's also interested in trinucleotide repeat disorders for neurodegenerative in general disorders, and in lipid nanoparticles.

Then, María Ortiz introduced herself as she's going to help from the administrative point of view with the coordination of this group. On the other hand, Alicia, Mª Ángeles and Ana, they come from the same group in Victoria. They are specialized in lipid nanoparticle systems, which they have used to conjugate plasmids, sRNAs, mainly for lysosomal disorders. As they are very interested in retina, there was a bit of talk about how is the lipidic nanoparticles delivered to the retina, which is in the vitreous.

At the end of the presentations, it was identified that compared to other types of tissues, in the case of brain and muscle, the main interest is focused on *in vivo* strategies, which are lagging the *ex vivo* strategies. Therefore, maybe this work is still defined at the preclinical level in *in vitro* models so that the subworking group can include information regarding *in vitro* delivery systems as well as *in vivo*, but not only *in vivo*.

Furthermore, it was discussed to consider the diversity of cell types within the brain and muscle because the different vectors may have different activity and tropism for the different cell types. IN fact, Cecilia suggested to start by drawing a map of what are the main delivery systems currently being used within the subgroup in a type of survey. Then Virginia said that from her experience in the previous cost action called ARTER, the problem is that the survey was very skewed towards only a few people, and this is a small working group. So that might not be a very good idea just to collect a survey that may fall off expertise, not have enough expertise within the subworking group, and maybe that the interaction with other subgroups like the subgroup on viral and non-viral delivery should be considered.

Virginia also suggested that one of the objectives of this subgroup could be to compare back to back the same delivery systems for different sequences, different nucleic acids, so see if they can be exchangeable.

Additionally, it was agreed that the creation of a template by the co-leaders of this working subgroup will be shared in the next meeting scheduled in a month's time. The aim of creating this template is that all members could briefly describe what is their expertise, what have they done and tested, what has worked, what has not worked, and what are their main needs, gaps, and challenges.

The frequency of the meetings of this subgroup
was also discussed and agreed in a monthly meeting to follow up. Here Karim suggested that this subgroup meets every two months. Later it was also mentioned that to include webinars in this subgroup.

At the end of the meeting, it was suggested to invite people even from outside COST Action to give talks on the gaps identified in order to increase the knowledge of all members. Furthermore, it was also discussed to write a reference document that could be turned into a review and how to do it.

Finally, Cecilia mentioned that she would like to invite her collaborators who produce lipid nanoparticles for muscle delivery.

WG6. TECHNOLOGICAL TRANSFER AND INDUSTRY.

The primary objective of this working group is to assess the current state of European facilities to establish guidelines to produce cost-effective GMP-compliant gene editing-based medicines. This includes identifying efficient sourcing strategies and promoting intellectual property management among partners. Additionally, WG6 aims to ensure the regulatory compliance of non-clinical models and methodologies used to evaluate efficacy and safety of various *in vivo* and *ex vivo* gene editing tools. It also seeks to facilitate the development of regulatory guidelines for the clinical translation of these gene editing technologies.

MAIN TASKS AND ACTIVITIES

1. Evaluation of the market and technology, conduct a report of technological surveillance where the evolution of technology, projections, scenarios, time to market and competitive advantages are included. To perform a market research study and competitive analysis on gene editing based medicines including the following tasks: (i) Market opportunity. Target niche; (ii) Definition of targeted market, sales by category (product, territory, class and market share (sales peak, defining at least three different scenarios), positioning, market drivers and barriers.
2. The purchase of market studies conducted by specialized independent analyst (*GlobalData, MedTrack, Datamonitor*) will be planned; (iii) Current and future potential competence identification, evolution, currently marketed competitive and developing products, competitive dynamics, potential collaborations, potential customers and suppliers; (iv) Identification of incentives for the

development of such products, trends in
the regulatory environment and other aspects that influence the potential market to be considered, (v) SWOT analysis.

3. To analyze regulatory requirements for the commercialization of gene editing based medicines. This task will perform an analysis of the requisites and steps to take in order to obtain authorization for the commercialization of gene editing based medicines in the relevant healthcare authorities. Guidelines to produce cost-effective and GMP compliant gene editing based medicines will be created.
4. Intellectual property rights management. To establish a broad strategy for the identification of Intellectual property and the protection of its rights (IPR) for future gene editing based medicine products, as well as possible technology transfer models for future products (licenses, co-development, corporate spin-off) considering the regulation framework.
5. An international workshop will be organized covering ATMPs regulation, with substantial experience and expertise in this field. It is planned to bring together international experts of the gene editing field to discuss the state-of-the-art in gene editing around the three regulatory pillars forming the basis for clinical use of ATMPs: 1) quality including manufacturing and control of the (investigational) medicinal product, 2) non-clinical testing in adequate animal models for efficacy and safety prior to clinical use and 3) clinical development including clinical protocol suitability (patient cohort, dosing, exclusion /inclusion criteria, etc.).
6. A white paper summarizing the outcome of the workshop and, if possible, formulating regulatory recommendations for clinical implementation of gene editing will be written and published. Selecting the appropriate non-clinical regulatory studies for GE tools is challenging. One problem is the species-specificity of some GE tools and the potential immunogenicity of nucleases. Thus, homologous models and the use of different complementary non-clinical models (in silico, in vitro or in vivo) is required. Also, for safety studies, appropriate tools must be validated to address tissue- specific off-target toxicity. For these purposes, we will work with WG3 and WG4 to establish the adequacy of non-clinical models and methods for regulatory approval of GE technologies.
7. To promote and participate in the development of GE guidelines for clinical translation.
8. The translation of GE technologies to the clinic presents many regulatory and ethical hurdles. We will work towards the development of clear international guidelines and will be in communication with European agencies and Scientific Societies to participate in the elaboration of the necessary GE guidelines for the

translation of these products to the patients, considering specific GE tools and delivery systems.

DESCRIPTION OF DELIVERABLES AND TIMEFRAME

- Patents application files.
- Industrial agreement.
- Formulation of guidelines and documents for translation of gene editing from bench to bed/market.
- Peer-reviewed articles analyzing different regulatory issues related to human genome editing.
- Formulation of guidelines and documents concerning regulatory aspects of new gene editing tools.

The timeline presented by figure 4 outlines the different meetings organized by Working Group 6 (WG6) since October 2023. It offers a chronological overview that enhances clarity regarding the collaborative efforts and progress made by WG6 during these months.

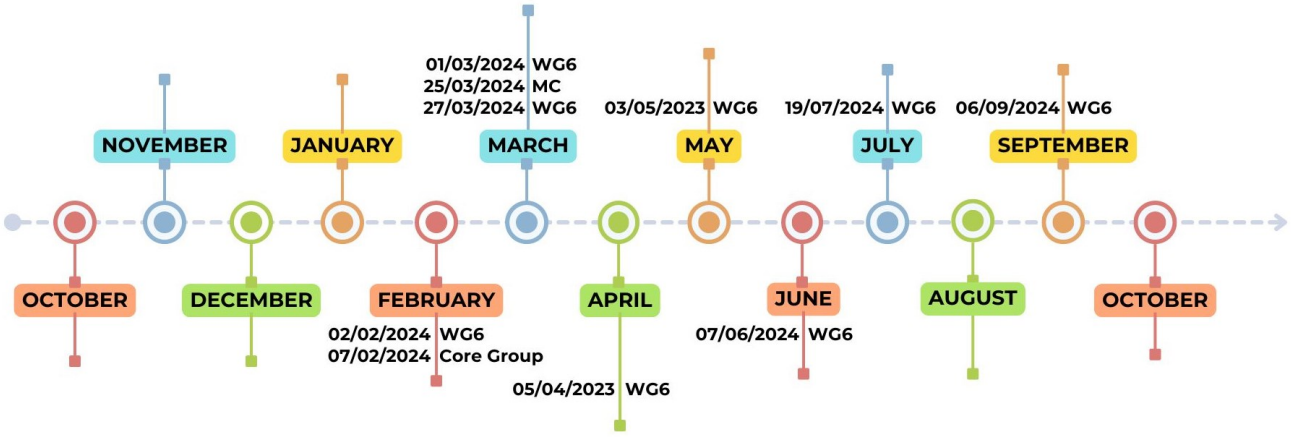

Figure 4. Meetings of WG6 since October 2023.

Unfortunately, the minutes from these meetings are not available.

WG7. DISSEMINATION.

The main objective of this WG is to disseminate the outcomes of the Action and integrate research and analysis findings to reduce knowledge fragmentation among partners. Additionally, it aims to promote popular science using social media.

MAIN TASKS AND ACTIVITIES

1. An Action website will be set up, including details of all Action members, ongoing activity and key documents.
2. The elaboration of a communication and dissemination plan.
3. The organization of several workshops and meetings with all WGs.
4. The dissemination of the activities and results using mainstream social media (Twitter, Instagram, LinkedIn) to reach non-specialized audiences.
5. Participate in national and international initiatives that aim to make science visible (Day of the Woman in Science, Day of the Rare Diseases, etc.).
6. Priming the interaction among research groups and patient associations.

DESCRIPTION OF DELIVERABLES AND TIMEFRAME

- A strategic plant for the liaison between networks members, work groups and external stakeholders.
- A proceeding booklet summarizing all the main conference of each WG.
- A friendly implemented Website with Action description, protocols, social media, measurable deliverables. (Dissemination: During the Action)
- White paper on best practice of clinical application of genome editing.
- Intervention in scientific and medical Assembly at EU Commission. (Dissemination: During the Action)

Displayed here in Figure 7 is a timeline depicting WG7 meetings scheduled within this timeframe. Although the meetings primarily focused on the organization of the Short-Term Scientific Missions (STSMs), members of WG7 have been present at most of the meetings held by the other working groups. They have participated as part of the team to gather ideas on dissemination.

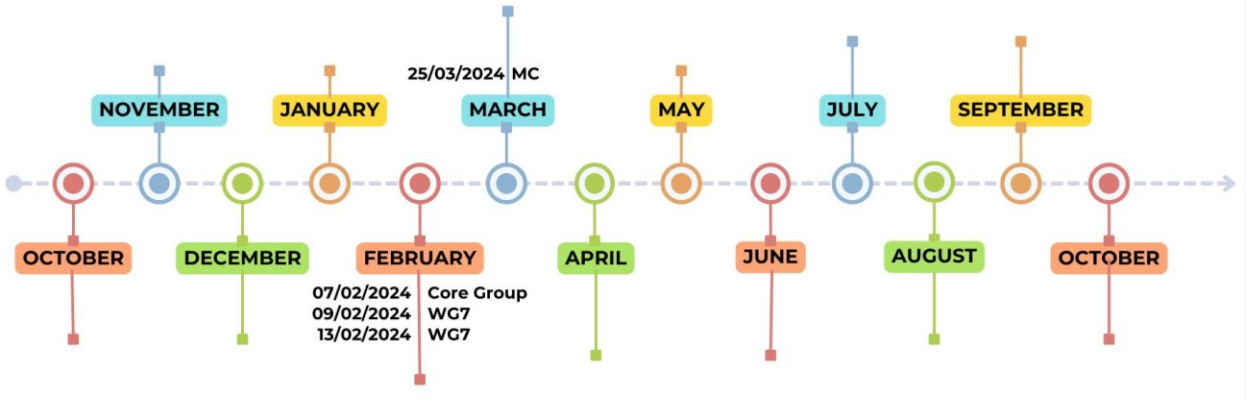

Figure 5. Meetings of WG7 since October 2023.

WG7 MEETING: STSM COORDINATION MINUTES - 09/02/2024

MODALITY: Virtual Meeting by Google Meet

DURATION OF THE MEETING: 52 minutes

PARTICIPANTS LIST (4 attendees):

1. Fatma Zehra
2. Karim Benabdellah
3. Francisco Javier Molina Estévez
4. María Ortiz Bueno

AGENDA

- Arrangement of the Short-Term Scientific Missions and Grants.
- Organization, decisions making.
- Setting time frames.
- Q&A.

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MINUTES

- It is agreed to contact Eduardo to have all the certificates of STSM ready once Fatma send the list of people to be included.
- Karim has commented on the budget for STSM and has proposed to start announcing them on website so people can see it. Referring to last year, there is algo a report to be uploaded where summaries of presentations could be included.
- Javier will announce next week the meeting in Cyprus, so it is important to announce too the week after the STSM. In addition to the report, people have been offered the opportunity to participate in talks and seminars.
- Paula Río needs to write the rules.
- The timeframe for people to apply is according to last year. Therefore, the call should be open soon, for example, at the end of February. Creation of an email was suggested to start establishing connections as well as thinking about possible hosting. It will be done as a pre-announcement on social media first.
- On Tuesday 13th February, Javier will do the precall and Fatma will send the general email.

WG7 MEETING: STSM COORDINATION MINUTES - 13/02/2024

MODALITY: Virtual Meeting by Google Meet

DURATION OF THE MEETING: 45 minutes

PARTICIPANTS LIST (4 attendees):

1. Fatma Zehra
2. Paula Río (Grant Coordinator)
3. Karim Benabdellah
4. María Ortiz Bueno

AGENDA

- Arrangement of the Short-Term Scientific Missions and Grants.
- Organization, decisions making.
- Setting time frames.
- Q&A.

MINUTES

- Opening of the call for all grants is in the middle of April.
- Paula has suggested to contact with some members of the different working groups (specially seniors and post-PhD) to select six people in order to create a committee.
- Abstracts should be accepted by firsts of October 2024.

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